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Researches in the Minute Anatomy of the Epithelia of the Kidney.

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RESEARCHES IN THE MINUTE ANATOMY OF THE EPITHELIA OF THE KIDNEY.*

By HENRY B. MILLARD, A. M., M. D.

R. Heidenhain † was the first to call attention to the presence of a peculiar rod-like or bacillated structure existing in the uriniferous tubules. He found this structure in convoluted tubules, in the ascending portions of the looped tubules, and in the intercalated tubules of the kidneys of mammals.

According to his view, the rodlets (Stäbchen) are plainly visible in the outer portions of the epithelia—that is, in those portions lying next the connective tissue, and he sometimes saw in torn epithelia the rods isolated. The same observer ‡ also first demonstrated with accuracy that the secretion of the salts is performed only in the tubules, in accordance with the views maintained by Bowman. Charcot # deduces from the experiments of Heidenhain with indigo-blue the conclusion that the secretion or elimination of this coloring matter takes place only in those portions of the tubuli uriniferi which are covered by the epithelia having the rods (épithélium à bâtonnets). Whether the secretion of the specific principles of the urine takes place in precisely the same fashion as the elimination of coloring matters, he regards as impossible of demonstration experimentally.

* Read before the New York Medico-Chirurgical Society, May 9, 1882.

† "Mikrosk. Beiträge zur Anat. und Physiologie der Nieren." Max Schultze's "Arch. f. mikr. Anat.," 10 Bd., 1874.

‡ "Versuche über den Vorgang der Harnabsonderung." Pflüger's "Archiv," 9 Bd., 1874, p. 1.

"Charcot on Bright's Disease," translated by Millard, New York, 1878, p. 23.

In a recent monograph by Charcot, *Leçons sur les Conditions Pathogéniques de l'Albuminurie*, Paris, 1881, he regards the *tubuli contorti* and the loops of Henle, particularly the ascending branches of the loops, as the real glandular part of the kidney. "They are," he says, "lined by an epithelium, thick, granulated, cloudy—in a word, glandular. They are enveloped in all parts by a dense capillary network, bathed, like themselves, in a lymphatic fluid." "These parts seem, then, in some respects, designed for the selection and concentration of the specific principles of the urine, urea and uric acid; it is in these parts, no doubt, that is formed the hippuric acid, which does not pre-exist in the blood."

Heidenhain, however, did not associate the rods with the process of secretion, for he observed a similar structure also in the smaller ducts of the parotid and submaxillary glands, the same formation in the latter structure being already known to Henle and Pflüger. In the acini of the glandula submaxillaris, and in the other acinous glands, he could not discern them.

E. Klein * asserts that he has observed that the rods or fibrils of Heidenhain, when looked at from the surface, are connected into a network, so that they are more probably septa of a honey-combed network seen in profile. What the intimate nature of these formations is, neither of the above-named authors attempts to explain. My own researches, I hope, will prove their nature, though as to their significance I have only suggestions to make. Since the reticular structure of all protoplasmic formations, including, therefore, epithelium, was demonstrated by C. Heitzmann, † the question has been, what the reticulum present in the protoplasm is. Unquestionably the two main properties of living matter are motion and production of its own kind. Both these properties are attributes of the reticulum within the protoplasm. As long as a protoplasmic body is alive and endowed with the property of amœboid motion and locomotion, the reticulum in it is never in a state of perfect rest. We constantly see changes in the configuration of the reticulum. We see that in a portion of the protoplasmic body the reticulum becomes very narrow, while in an opposite portion it is simultaneously widened, especially so when a prolongation of the body, a pseudopodium, is pushed out. In such a flat offshoot, or false leg, the reticulum, may be stretched to such a degree that the projection looks homogeneous, as if destitute of any structure.

The writer above quoted claims that the narrowing of the reti-

* "Atlas of Histology," London, 1880.

† "Untersuchungen über das Protoplasma." "Sitzungsberichte d. kaiserl. Akad. d. Wissensch. in Wien," 1873.

culum is the state of contraction which is an active property belonging to it. The stretching, on the contrary, represents the state of extension which is merely passive, due to the pressure of the liquid pushed out from the contracted portion into that at comparative rest, this contracted portion being immediately after extended.

The foregoing is tenable only if we admit the presence of an investing layer around the protoplasmic body which prevents the liquid filling the meshes from escaping outward. The flat investing layer is claimed to be identical in its nature with the mass composing the reticulum proper. It is maintained, also, that the reticulum at any time, and almost instantaneously, may be transformed into a flat layer, as is the case in the formation of an investing layer around a vacuole. *Vice versa*, the flat layer almost instantaneously may fall back into the reticular structure at the moment of disappearance of the vacuole. This continuous change of shape and place of the reticulum is a positive proof of its being living matter. S. Stricker,* among the most recent observers, describes the reticular structure and its changes as follows:

"The interior of the cell-bodies undergoes manifold visible variations. One of the most remarkable instances is furnished in the saliva corpuscles. The assumption that a so-called molecular motion takes place in the saliva corpuscles is erroneous. The granules seen with insufficient amplifications are transverse sections of trabeculæ. The saliva corpuscle is traversed by a sharply marked trabecular structure, which, so long as the corpuscle is fresh, executes lively wavy motions. The waving gradually ceases on addition of solutions of salts in certain concentration, and the reticular structure disappears. The waving is now replaced by very slowly formed changes in the interior mass."

A second proof of the reticulum being the living matter proper rests upon the fact that, both in normal and in morbid processes, the new formation of corpuscular elements starts from the points of intersection in the reticulum. This so-called endogenous new formation of living matter is especially plain in the inflammatory process invading epithelial formations. Here, it is important to note, the reticulum at first becomes coarse, next it coalesces into lumps, which, being at first homogeneous, in turn assume a reticular structure themselves, and now represent so-called inflammatory or pus corpuscles. These corpuscles at first remain in connection with the neighboring reticulum by means of delicate filaments, which

* "Mittheilung über Zellen und Grundsubstanzen." "Med. Jahrbücher," 1880.

are portion and part of the reticulum. Later, when the pus corpuscles which have originated in the interior of an epithelium become extruded from its interior, the newly formed corpuscles represent pus corpuscles.

In conducting my researches, I have studied the kidneys of the rabbit, pig, dog, and man, all of them being preserved and hardened in a solution of chromic acid. I have, therefore, no observations to report upon the form changes of the epithelia, but have studied the changes in the interior structure of the epithelia in the inflamed human kidney as they appear in chronic croupous, in chronic interstitial nephritis, in waxy degeneration of the kidney, in fatty degeneration, and in chronic interstitial nephritis with acute recurrence. These investigations enable me to maintain that the reticular structure of the epithelium of the kidney *is really a formation of living matter.*

Upon closely examining the epithelia of the tubuli uriniferi in the kidneys of the above-named animals, we readily perceive, with comparatively low powers of the microscope (400 or 500 is sufficient), the presence of rod-like formations in the epithelia of the *tubuli contorti*, in the irregular tubules, in the ascending branch of the looped tubules, and in the intercalated tubules, entirely in accordance with Heidenhain's assertions, although he does not include the kidneys of the pig.

The drawings of the rodlets, as given by Heidenhain in Max Schultze's "Archiv," and copied by Klein and other writers, give an exaggerated idea of the real appearance of the rods. Even under a high power they are never so large as in the drawings, and seldom present the straight, regular, and symmetrical appearance there represented. The accompanying drawing (Fig. 1) more nearly represents their average appearance under a power of 1,200.

I have found them in the healthy kidney as follows:

In man, in the ascending tubule, power 1,200.

In the rabbit, power 500 to 600, in convoluted, in ascending, and in irregular tubules. Also (never before mentioned) in a portion of the descending tubules.

In the pig, in the convoluted and irregular tubules; and in the same tubules, and narrow tubules, in which the rods are very faintly shown, of the pup.

The pale, flat epithelia of the looped tubule proper do not, as a rule, exhibit the rods. The columnar epithelia of the collecting tubules, on the contrary, which are distinctly imbricated, especially in the kidney of the dog, exhibit the rods more or less plainly. The

columnar epithelium of the rabbit does, however, show them. High powers (1,000 to 1,200) of the microscope corroborated the

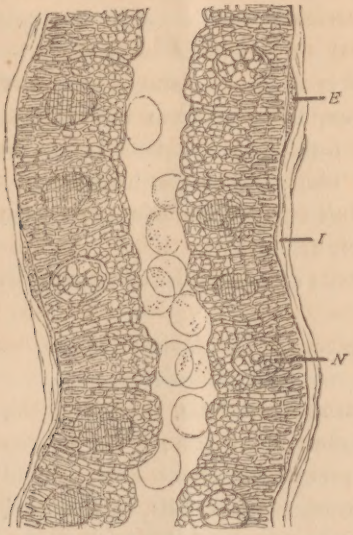

FIG. 1.—CONVOLUTED TUBULE FROM THE KIDNEY OF A RABBIT. (Longitudinal section—magnified 1,200 diameters.)

N, nucleated columnar epithelium, showing the rods; *E*, endothelia; *I*, interstitial connective tissue, producing the basement layer.

views of Klein—namely, that the rods are connected with a reticulum by means of delicate filaments inosculating both with the wall of the nucleus around which the rods are located, and also with the delicate reticulum in the inner portion of the epithelia, next to the caliber, where the rods are usually absent. It is striking how the thickness of the rods differs in the different epithelia of the same animal's kidney. Sometimes they are very thin, beaded poles, with quite distinctly marked interstices between them. In this case the connecting filaments, running almost at right angles from rod to rod, are easily discernible. At other times the rods are rather bulky formations, having but extremely narrow interstices between them. In this instance the connecting filaments, as a matter of course, are very short, and not easily seen. In a third instance the outermost portion of the epithelium is a compact or homogeneous mass, in which no rods can be observed at all.

Another striking feature is the great variety of appearances exhibited by the cement-substance. Sometimes this is plainly marked at regular intervals between the epithelia. Then the transverse connecting filaments, the formerly so-called thorns, are plainly visible. At other times hardly any trace of cement-sub-

stance is seen, but the reticular structure is present in a nearly uniform distribution throughout the epithelial layer. S. Stricker (*loc. cit.*) was the first who observed these same varieties in the appearance of the cement-substance in the epithelial layer of the cornea; also, that the nucleus varies greatly in the degree of distinctness in which it comes to observation. Where the rods are slender, the nucleus, as a rule, is well defined; where, on the contrary, they are bulky, the nucleus is, on an average, not very plainly marked. The sharpest definition of the nucleus is furnished by the flat epithelia of the looped tubules in which the rods, as before mentioned, are absent.

In inflamed kidneys of man I have repeatedly found the rods as follows:

1. In chronic interstitial nephritis:
   a. In the convoluted tubules.
   b. In the straight tubules.
2. In acute croupous nephritis:
   a. In ascending tubules.
3. In chronic croupous nephritis, in the straight tubules.
4. In chronic croupous nephritis with waxy degeneration, cross-sections of ascending tubules show the rods rather enlarged. Also in straight tubules in the pyramid of the same kidney.
5. In chronic croupous nephritis with acute recurrence, in cross-sections of the convoluted tubules.
6. In fatty degeneration of the kidney, in cross-sections of the convoluted tubules. The rods here showed fat globules. The connective tissue was thickened.

In these specimens the rods of the epithelia throughout the tubules are clumsy and bulky, the whole reticulum being enlarged, rendering the epithelium, with low powers of the microscope, coarsely granular. In many instances the rods are not discernible, as, in their place, a coarsely granular mass is present, pervading the whole epithelial body; or else the innermost portion of the epithelium looks coarsely granular, the outermost portion, on the contrary, being homogeneous and shining. I have repeatedly seen in acute interstitial nephritis even the looped tubules, which in this situation were considerably increased in bulk, provided with a coarsely granular reticulum—nay, even with an indistinct rod-like structure. All these features become still more prominent by staining the specimens with the chloride of gold after they have been soaked and washed for several days in distilled water. This reagent, in a half-per-cent. solution, brought in contact with the specimens for forty minutes, renders sections from the normal kidney of a brown

violet hue, slightly increasing the distinctness of the reticular structure of the epithelia. In the inflamed kidneys of man, the epithelia of a great many of the ascending, irregular, and convoluted tubules, upon being stained with the chloride of gold, as above described, became dark violet. With higher powers of the microscope we can ascertain that it is the coarse reticulum, the bulky rods, and the homogeneous masses sprung from coalescence, as it were, of the rods, which exhibit the deepest gold stain.

As it is the tubuli uriniferi which have the rod-like structure, which in Heidenhain's experiments with indigo sulphate are the only ones which are colored by it, so in the inflamed kidney it is only these tubules that become colored by the gold. It seems reasonable to suppose, from the effect of these reagents, that the epithelia with rods, perhaps by virtue of their having more living matter and a more bulky reticulum, are of most importance in secreting or forming the extractive matter of the urine.

Numerous attempts to produce the stain with the gold in the healthy kidney of the dog, pup, rabbit, and pig, were ineffectual in rendering the rods plainer than in the unstained condition.

In the inflamed kidneys, in which the violet coloration was produced, no doubt the reticulum of the epithelia, owing to the in-

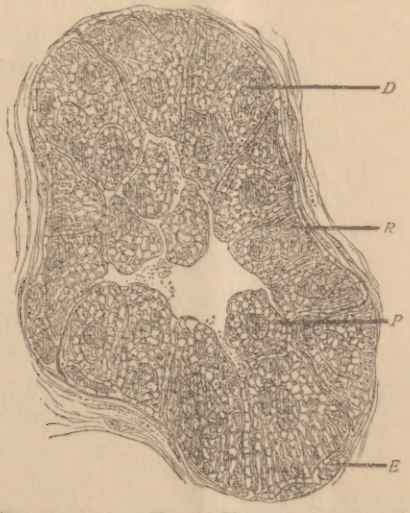

FIG. 2.—CONVOLUTED TUBULE FROM A HUMAN KIDNEY AFFECTED WITH ACUTE CATARRHAL (INSTERSTITIAL) NEPHRITIS. (Oblique section—magnified 1,200 diameters.)

P, inflammatory corpuscle, sprung from the division of an epithelium; *D*, cluster of inflammatory corpuscles, sprung in the same manner; *R*, rods of cuboidal epithelia, still recognizable; *E*, endothelia, increased in size and number.

flammatory process, was considerably increased in bulk. The most marked violet stain was exhibited by a number of the convoluted

tubes and by irregular and ascending tubules. We know that living matter is considerably increased in amount in the inflammatory process, and are justified, consequently, in maintaining that the reticulum and the rod-like formations within the epithelium, being part of the reticulum, are formations of living matter.

As to the significance of the rods, it may be inferred from the statements I have made that they are in close relation with the process of secretion. Obviously, the stream of liquid running from the neighboring blood-vessels through the epithelia toward the liquids contained in the caliber, and *vice versa*, will be facilitated by an elongated arrangement of the reticulum—i. e., the rods. In a state of comparative rest the rods lie close to each other—nay, are coalesced into homogeneous masses. In this condition the cement-substance between the epithelia is best marked. In full activity of the epithelium, on the contrary, the rods will be very distinct, will stand farther apart, and the cement-substance between the epithelia will, in consequence, become indistinct.

THE ENDOTHELIA OF THE URINARY TUBULES.

While investigating the peculiarities in the structure of epithelia of tubuli uriniferi in their normal condition, I often observed the presence of flat, spindle-shaped bodies between the basis of the epithelia and the adjacent so-called structureless membrane of the tubule. These spindle-shaped bodies doubtless correspond to those flat, nucleated formations which cover the inner surface of the structureless layer in nearly all epithelial—i. e., glandular—formations. By most observers they are regarded as endothelia belonging to the connective tissue subjacent to the epithelial layers. V. Czerny was the first one to bring them to view in other tissues, which he did by staining the specimens with the nitrate of silver; and C. Ludwig,* also by the silver stain, first indicated their presence in the urinary tubules. He does not fully describe them, but alludes to them as follows. Speaking of the basement membrane of the tubuli uriniferi, he says: "In general, the basement membrane appears to be homogeneous, and can not be further divided; but occasionally a nucleus can be brought into view in its substance by carmine; and in some instances, and for short distances, the same appearances occur in the tortuous canals, when treated with nitrate of silver, as are presented by the blood and lymph capillaries under the same condition." "The basement membrane is as clear as glass, elastic." "The shape of the nucleus is usually the same in all instances, being

* "Hand-book of Histology," by S. Stricker, London, 1874.

spherical, sharply defined, and with numerous granules scattered through its substance."

Such an endothelial layer, present in all varieties of the urinary tubules, is best visible in the front view of the structureless membrane, where the epithelium is stripped off. Here the endothelia are comparatively large, irregularly polyhedral bodies, with distinct central nuclei. The nucleus has a plainly marked shell, containing in its interior a few small nucleoli, the nuclei being mostly of oblong shape. In the body of the endothelium a delicate reticulum is seen with very minute nodulations. Each body is separated from all its neighbors by a delicate light rim of cement-substance, which is traversed at right angles by extremely minute filaments or thorns. In side view, obviously, these bodies will exhibit a spindle-shape, the broadest portion of the spindle corresponding to the central nucleus.

If the views of recent observers are correct—namely, that the structureless layer, synonymous with the hyaline or basement layer, is an aggregation of endothelia infiltrated with elastic substance—this view may also be applied to the structureless membrane of the urinary tubules. In normal kidneys I failed to discover nuclei in the structureless layer proper, which would indicate their construction of former endothelia. In inflamed kidneys, on the contrary, no doubt was left as to the fact that the structureless layer is composed by a number of closely attached, in part nucleated, endothelia.

I have found the endothelia repeatedly in the inflamed kidney in chronic croupous and in chronic interstitial nephritis, in acute interstitial and in acute croupous nephritis, and in fatty and waxy degeneration of the kidney. I have found them most frequently in the ascending, descending, and convoluted tubules. I am not aware that any observer has heretofore recognized their existence as having a pathological significance.

In the inflamed kidney the endothelial layer beneath the epithelial is always more marked than in the normal kidney. In chronic catarrhal (interstitial or desquamative) nephritis, all the tubules that have lost their epithelial investment invariably show an investment of endothelia.

This, in the transverse section of the tubule, is characterized by the presence of flat, irregularly spindle-shaped bodies, which are always more coarsely granular than in the physiological condition. Their nuclei are also more coarsely granular, sometimes homogeneous. The flat shape, the large size in the frontal diameter, and the construction of the nuclei serve for an accurate contradistinction

to epithelia. I have failed in obtaining specimens indicative of a new formation of epithelia after the loss of the original epithelial investment.

FIG. 3.—CONVOLUTED TUBULE FROM A HUMAN KIDNEY AFFECTED WITH CHRONIC CATARRHAL (DESQUAMATIVE) NEPHRITIS. (Oblique section—magnified 1,200 diameters.)
C, caliber, widened by loss of the epithelia; *E*, endothelia, increased in size and number; *F*, interstitial fibrous connective tissue, with augmented plastids.

It may be admissible to assume that the enlarged endothelial layer serves (at least to some extent) as a substitute for the lost epithelia. In tubules whose epithelia, as in chronic catarrhal nephritis, are transformed into inflammatory or medullary corpuscles, the new formation of such structures also starts from the endothelia. The final result in this instance is known to be the destruction of the tubule and its replacement by newly formed connective tissue—a condition which is known by pathologists as cirrhosis of the kidney.

[Since writing the rough outlines of this article, I have recognized for the first time well-marked endothelia in the urine in a case of advanced chronic croupous nephritis with fatty degeneration. I found a cluster of three or four of these surrounded by free fat granules.]

Still more plainly marked are the endothelia in croupous (parenchymatous) nephritis. In fact, the appearances seen in urinary tubules where casts have just formed could not be explained unless by the presence of endothelia.

We do not yet know what the mass composing a cast really is. This much, however, is certain, that casts are proteinates and forma-

tions of an albuminous or fibrinous exudate sprung from the blood-vessels. This exudate, before it reaches the central caliber of the tubule, necessarily must saturate the intervening epithelia, whose structure is completely destroyed by this process. It is not my purpose to dwell upon the origin of casts, but, from what I have seen, I can not concur with Oedmansson * in the opinion that every cast should be regarded as a product of secretion furnished by the epithelium. I am sure that the epithelia perish in the formation of the cast. Neither can I agree with Charcot † in the opinion that some (certain granular) casts are made up of broken-down epithelial cells, others (hyaline and some granular) of an albuminous substance, while epithelial casts are agglomerations of epithelial cells more or less altered.

Bartels ‡ insists that, in every case in which he has examined microscopically thin sections of diseased kidneys whose tubules were blocked by the dark granular casts, the tubules invariably exhibited an epithelial lining, reconciling this fact with his view by admitting that the theory of Key and Bayer, that the epithelium thus shed is rapidly reproduced, may be correct.

From my observations, it is obvious that the last three writers have regarded the endothelia, as I have described them, as epithelia.

Nevertheless, whenever we find a cast within a tubule, especially in transverse sections of the tubule, we almost invariably see a wreath of irregularly spindle-shaped, partly nucleated bodies, which I am sure are nothing but the lining endothelia of the structureless membrane.

This wreath around the cast may be easily recognized by any good observer. Dr. Alfred Mayer,# of New York, gives illustrations of these wreaths, which evidently are drawn with the greatest accuracy; but he does not realize at all their character or significance, for he suggests that they are constructed either of remnants of the former epithelia, of which a large portion has been destroyed in the formation of the cast, or that they may be newly formed epithelia. In both these views he is mistaken. The epithelia are certainly gone, entering in a considerably swollen condition the mass of the cast; but what is behind the cast is not newly formed

* Bartels, von Ziemssen's "Cyclopædia," vol. xv, p. 84.

† Charcot, "Bright's Disease." Millard's translation, New York, 1878, pp. 29, *et seq.*; quoted by Tyson.

‡ Bartels, *op. cit.*, pp. 84–86; quoted by Tyson, on "Bright's Disease."

"Untersuchungen über acute Nierenentzündung." "Sitzungsb. d. Akad. d. Wissensch. zu Wien," 1877.

epithelia, but merely the endothelial investment of the structureless layer, considerably increased in size. Not infrequently we see

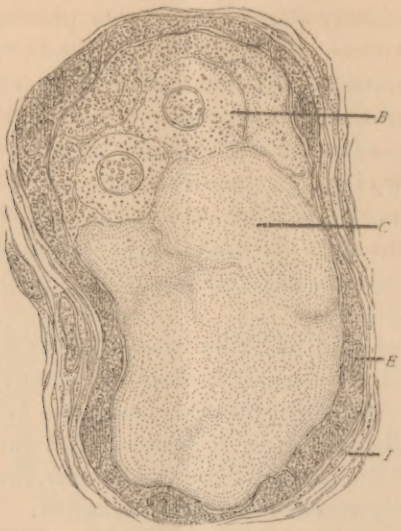

FIG. 4.—CONVOLUTED TUBULE FROM A HUMAN KIDNEY AFFECTED WITH ACUTE CROUPOUS NEPHRITIS. (Oblique section—magnified 1,200 diameters.)
C, hyaline cast; *B*, swollen and disintegrated epithelia participating in the formation of the cast; *E*, wreath of endothelia; *I*, interstitial connective tissue.

widened urinary tubules, as a rule, of the convoluted variety, entirely destitute of epithelia; or we see such tubules containing a cast broader in its diameter than the caliber of the tubule would be if the epithelial layer were present. The latter feature is explicable by the fact that casts may be carried into tubules far distant from the place of their origin—into tubules, besides, which have been previously deprived of their epithelia. There is no cogent necessity whatever for the conclusion that casts may form in tubules after these have lost their epithelia. In neither of these instances shall we ever miss the endothelial investment, although this is often found in a mutilated or imperfectly developed condition.

The results of my researches may be summed up in the following statements:

1. The rods discovered by Heidenhain in some varieties of the tubuli uriniferi are part and parcel of a reticulum present within every epithelium.

2. The reticulum, including its elongated rod-like formations, is the living matter proper.

3. The relation of the rods to the rest of the reticulum of an epithelial body varies greatly, the variation probably being due to different stages or degrees of secretion.

4. The reticulum, including the rod-like formations, in the inflammatory process, both in catarrhal and croupous nephritis, gives rise to a new formation of living matter, which results in the new formation of medullary corpuscles or pus corpuscles.

5. The structureless membrane is lined by flat endothelia lying between it and the basis of the epithelia of the urinary tubules.

6. In nephritis the endothelia become considerably enlarged, and in catarrhal, as well as in croupous nephritis, they line the urinary tubules after the epithelia have been shed or lost; they surround the cast in croupous nephritis after the epithelia have perished in the formation of the cast.